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The studies were designed to provide a basis for developing a relatively rapid mammalian test system for lead, to provide information on intestinal absorption, routes of excretion, and rates of transfer to neonates, and to determine the usefulness of trace-element content of feces, urine, blood, hair, and other tissues for estimating exposure. As rodents are endemic to most areas of interest, the laboratory rat was used as the biological monitor. As resident avian species are also readily available in most areas of interest, a study was undertaken to determine if Japanese quail could function as reliable indicators to track the movement of pollutants from source to receptor.

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February 1980

STUDIES TO DETERMINE THE ABSORPTION AND EXCRETION DYNAMICS OF LEAD

by

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FOREWORD

Protection of the environment requires effective regulatory actions based on sound technical and scientific data. The data must include the quantitative description and linking of pollutant sources, transport mechanisms, interactions, and resulting effects on man and his environment. Because of the complexities involved, assessment of exposure to specific pollutants in the environment requires a total systems approach that transcends the media of air, water, and land. The Environmental Monitoring Systems Laboratory at Las Vegas contributes to the formation and enhancement of a sound monitoring-data base for exposure assessment through programs designed to:

- develop and optimize systems and strategies for monitoring pollutants and their impact on the environment
- demonstrate new monitoring systems and technologies by applying them to fulfill special monitoring needs of the Agency's operating programs

This report summarizes information useful in assessing inorganic pollutant exposure from pollutant-induced biological responses. The report deals primarily with the absorption and excretion dynamics of lead in representative biological monitors. For further information contact the Exposure Assessment Division.

George B. Morgan
Director
Environmental Monitoring Systems Laboratory
Las Vegas

ABSTRACT

This is a summary report of studies designed to provide a basis for developing a relatively rapid mammalian test system for lead, to provide information on intestinal absorption, routes of excretion, and rates of transfer to neonates, and to determine the usefulness of trace-element content of feces, urine, blood, hair, and other tissues for estimating exposure. As rodents are endemic to most areas of interest, the laboratory rat was used as the biological monitor. As resident avian species are also readily available in most areas of interest, a study was undertaken in conjunction with Dr. F. W. Edens et al. of North Carolina State University, Raleigh, N. C., to determine if Japanese quail could function as reliable indicators to track the movement of pollutants from source to receptor.

Results indicate that 3 days following a single oral administration of lead-210 to rats, about 0.2 percent of the dose was excreted in the urine and 70 percent in the feces. After 12 days the percentages had increased to about 0.3 and 90 percent respectively. The main deposition sites were liver, 0.008 percent, and bone, 0.04 percent, of the oral dose.

To ascertain the role of bile in the excretion of lead, the bile ducts of young adult rats were ligated and comparisons of excretion rates were made with rats having intact ducts. During the 3 days following oral administration of lead-210, about 2 percent of the dose was accumulated in the bile, whereas, over the same time period, about 20 percent of an intravenous dose was accumulated in bile. This would indicate that biliary excretion is a main route for removing absorbed lead. Excretion curves for urine and feces indicate the primary difference between rats with intact bile ducts and those with ligated bile ducts was the increased percentage of lead excreted in the feces of intact rats.

Bone was again found to be the main deposition site for lead, regardless of the state of the bile duct. Placental and milk transfer of stable lead occurred in all animals. Assessing exposure to lead by means of collecting hair samples appeared to be a valid method provided the physiological state of the animal was considered. During pregnancy and lactation, or rapid growth, rats do not appear to incorporate as much lead in their hair as they do at other periods of time.

Using resident avian populations as reliable biological indicators of increased lead in the environment appears promising. Dependent on the amount of lead in the diet, lead concentrated in all portions of the quail egg, but mainly in the shell. Analysis of quail femur samples yielded similar results.

This report covers a period from 1976 to 1979 and work was completed in 1979.

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Since our Laboratory was interested in correlating the dose to concentration values in the egg shells and femurs, a collection of Japanese quail eggs and femurs were kindly sent to us by Dr. F. W. Edens et al. of North Carolina State University, Raleigh, North Carolina, for analysis.

INTRODUCTION

A considerable body of information has been published on the environmental aspects of lead (Barth et al., 1973; National Academy of Science 1972; Assoc. Comm. Sci. Criteria Environ. Qual. 1973; Task Group on Metal Toxicity 1976; World Health Organization 1976; and U.S. EPA 1977). The effects of lead on laboratory animals have been extensively investigated for the purpose of extrapolating this information to hazard evaluation in humans. There is relatively little information, however, on exposure/dose studies to develop convenient biomonitors for assessing human exposure.

Avian species resident in the area being monitored have been considered by several investigators as possible indicators of lead pollution (Ohi et al., 1974; Tansy and Roth 1970). Tansy and Roth, reporting on a small number of pigeons, showed that there was a substantial increase in the lead content of various organs and tissue from pigeons obtained in urban Philadelphia as compared to those obtained in rural Pennsylvania. Ohi obtained a large number of pigeons from locations in both downtown and suburban Tokyo. Determination of lead levels in blood, femur, and kidney demonstrated highly significant regional differences. Other authors have reported the presence of lead in tissue and eggs from migratory bird populations (Lincer and McDuffie 1974; Martin and Nickerson 1973). However, little information is available on the relationship of intake to tissue levels.

Studies show the gastrointestinal tract to be the main pathway for the excretion of heavy metals (Witschi 1964; Castellino et al., 1964; Cikrt 1972; Stanley et al., 1971). Three routes exist for transport of heavy metals into the intestinal lumen, namely ingestion, secretion by the intestinal mucosa, or by the liver into the bile. Some contradictory reports question whether the bile or intestinal wall is the significant pathway for lead excretion (Karhausen 1973).

The present studies were undertaken in order to work out the problems associated with determining the mammalian samples to be collected, and with their subsequent analyses to estimate dose and potential hazard to the environment.

CONCLUSIONS AND RECOMMENDATIONS

Hair samples from rodents appears to be a valid medium for obtaining information on ingested lead without having to sacrifice the animal. Care must be taken to wash the hair thoroughly before analysis. It is also important to ascertain the physiological state of the animal when selecting individuals to represent the population as a whole. Information on lead derived from hair or bone samples of lactating animals cannot be considered representative because metabolism is altered during lactation.

Bile fluid appears to be the major excretory route of absorbed lead in rats. The most concentrated and earliest indicator of lead ingestion is the bile. Therefore, a quick test procedure for lead in bile would be useful for field testing, and bile should always be sampled for early detection of lead absorption.

Based on the results of the avian portion of the study, further investigation into the feasibility of using egg shells of resident species for determination of trace element pollutants would be advisable.

EXPERIMENTAL PROCEDURES

RODENT MONITORING

Excretion and Tissue Distribution

A preliminary range-finding study to determine the relatively long term excretion and tissue distribution of lead by rats utilized adult, 100-day old, 268-gram (g) female Wistar rats. Following fasting for 12 hours, the rats each received a single oral dose containing 4 microcuries (μCi) of lead-210 (^{210}Pb) as the nitrate plus 50 milligrams (mg) of stable lead nitrate per kilogram (kg) of body weight. The ^{210}Pb content used in these studies was obtained as lead nitrate [$Pb(NO_3)_2$] in 3N nitric acid (HNO_3) solution. The specific activity was 62.0 curies per gram (Ci/g) Pb and the purity was >99 percent. The doses, aliquoted by volume measurements of known dilution, were verified following administration by placing random doses in volumetric flasks and counting aliquots of the doses.

The rats were housed in stainless steel metabolism cages and maintained on commercial laboratory rat chow and deionized water. Total urine and feces excretions were collected daily, weighed, and analyzed by gamma spectrometry. Twelve days following dosing, the rats were sacrificed by anesthetic overdose, and blood was removed by means of cardiac puncture. Tissue samples were removed, weighed, and placed in scintillation vials containing formaldehyde. The ^{210}Pb content was subsequently determined by counting the samples.

All samples were counted for up to 40 minutes using a NaI(Tl) well crystal connected to a single-channel analyzer. Sample collection was discontinued when the counting errors exceeded 30 percent.

Biliary Excretion of Lead

To elucidate the mechanism by which lead is transferred to the intestine by ligation of the bile duct of rats to measure the difference in the excretion of lead by ligated and intact rats, two groups of 16 female Wistar rats, 51-day-old young adults, weighing 150 g, were used. A summary of rat treatments is shown in Table 1.

The common bile duct of six animals in each group was ligated. Food was withheld for 12 hours prior to surgery. The rats were anesthetized with Fluothane® and the bile duct was ligated at a point 5 or 6 millimeters (mm)

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TABLE 1. TREATMENT OF RATS

No. of Rats	Condition of Bile Duct	Method of Administration	Dose
2	Ligated	Control	None
4	Intact	Control	None
6	Ligated	Oral	4μCi ^{210}Pb + 50 mg/kg Body Wgt Pb $(No_3)_2$
6	Intact	Oral	4μCi ^{210}Pb + 50 mg/kg Body Wgt Pb $(No_3)_2$
2	Ligated	Control	None
2	Intact	Control	None
6	Ligated	Intravenous	2μCi ^{210}Pb + 10 mg/kg Body Wgt Pb $(No_3)_2$
6	Intact	Intravenous	2μCi ^{210}Pb + 10 mg/kg Body Wgt Pb $(No_3)_2$

below the hilus at the liver and just above where the pancreatic tissue no longer surrounded it. A surgical procedure described by Lambert (1970) was modified for use in this procedure. One hour after ligation of the bile duct, when the animal had recovered from the anesthetic, ^{210}Pb and stable lead were administered orally by means of a dosing needle and syringe or intravenously via the tail vein.

The rats were placed in stainless steel metabolism cages and total urine and feces samples were collected every 12 hours. Samples were collected in the same manner as the preceding group. Three days following administration of the radionuclide, the animals were weighed and sacrificed, and blood was removed by cardiac puncture. Tissue samples were collected and analyzed in the same manner as in the previous study.

Placental and Milk Transfer of Stable Lead

Fifteen recently impregnated Wistar rats were obtained. Samples of hair and blood were collected to determine baseline lead levels. On the ninth day of gestation, all of the pregnant rats, including the controls, were weighed and orally dosed with 50 mg/kg Pb $(NO_3)_2$ in water solution. Thereafter all drinking water supplied to eight of these rats contained 50 parts per million (ppm) of lead as lead acetate. The remaining seven pregnant rats received deionized water. Daily records of water intake were kept for all animals.

A total of 112 offspring were born to 10 pregnant rats. Of that total 67 were born to dams receiving chronic doses of lead as lead acetate, while 45 were born to dams receiving a single acute dose. The average litter size for dams receiving chronic lead was 11.2 with a standard deviation of ±2.3, while that of dams receiving a single chronic dose was 11.3 ± 1.5. The distribution of offspring is shown in Table 2. At parturition, two animals from each litter were sacrificed for whole body analysis of lead content. The litters were reduced to contain no more than six offspring. Blood and hair samples were collected from all dams at this time.

Hair and blood were collected from the dams and young upon weaning and at monthly intervals. Also upon weaning, two young from each litter were sacrificed for whole body analysis of lead content. Two young in each litter received water containing lead and two received deionized water. The dams remained on their previous diet and hair and blood samples were taken at monthly intervals from both dams and young.

Following parturition and about 12 days after initial dosing, four litters (24 offspring) of the rats not receiving lead in water were cross-fostered with lactating rats receiving lead daily. Four litters from rats receiving lead daily were cross-fostered with lactating rats not receiving lead, and 36 remained with their natural mothers. At weaning, 21 days following birth, 5 of the litters were placed on the same diet as the adults with water containing 50 ppm lead as lead acetate available ad libitum, and 5 litters received an identical diet but utilizing deionized water.

Hair samples collected during this study were removed using electric clippers. The clipper heads were rinsed with dilute HNO_3, then with distilled deionized water. The hair was stored in HNO_3-washed polyethylene bottles. The hair was washed using the procedure described by Clarke and Wilson (1974). This consisted of washing the hair twice in deionized water, followed by two detergent washes, and two deionized water rinses. The hair was then soaked twice in acetone, drained, and placed in a hot, saturated ethylene diaminetetraacetic acid (EDTA) solution, and allowed to soak for 5 minutes. The hair was then drained, rinsed in double-distilled deionized water, and drained. The EDTA soak was repeated and the rinsing procedure was repeated twice. The drained hair was covered and dried at room temperature. The hair was stored in HNO_3-acid washed, double-distilled deionized water-rinsed polyethylene bottles until weighed and wet ashed.

The tissue samples were removed using stainless steel instruments, weighed, and stored in acid-washed and rinsed polyethylene bottles, and stored at 0°C or below until wet ashed.

The samples were completely ashed in hot concentrated HNO_3 acid. Ultrex® (redistilled, high purity nitric) acid was used to minimize error due to introduced lead. Borosilicate glassware was used throughout the analysis. The glassware was cleaned by being soaked overnight in 1:1 HNO_3 acid, rinsed with water, and soaked overnight in distilled water.

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TABLE 2. DISTRIBUTION OF OFFSPRING

Number of Offspring	Treatment of Offspring				
	Cross-fostered to lactating females receiving lead (lead in milk)	Not cross-fostered	Cross-fostered to lactating controls (no lead in milk)	Weanlings on water containing lead	Weanlings on water containing no lead
Born to dams not receiving lead in water (controls):					
13	--	X	--	--	X
12	X*	--	--	X	--
10	X	--	--	X	--
Born to dams receiving lead in water:					
11	--	X	--	X	--
9	--	X	--	X	--
8	--	X	--	X	--
14	--	X	--	--	X
13	--	X	--	--	X
12	--	--	X	--	X
10	--	--	X	--	X

* Each X represents one litter of rats.

Following digestion, the resulting solution was analyzed for lead by atomic absorption spectrophotometry. The sensitivity of the analysis was about 0.11 micrograms per milliliter (μg/ml). The results were checked by using the method of standard additions.

Analysis of Lead in Blood

In a search for alternate methods to determine lead uptake, the ZnP (zinc protoporphyrin) Model 4000 Hematofluorometer® was evaluated. A preliminary study was conducted to determine whether induced lead toxicity in laboratory rats can be detected by the instrument, which is available commercially for use in lead toxicity screening clinics. Lead inhibits heme synthase, an enzyme critical to the conversion of protoporphyrins to heme. Lead toxicity results in increased blood levels of protoporphyrins which combine with zinc to form the fluorescent molecule, zinc protoporphyrin. The Hematofluorometer is designed to detect zinc protoporphyrin (ZnP) by front surface illumination of a drop of whole blood. Fluorescent wavelengths emitted by the ZnP molecules are subsequently detected by a photomultiplier tube. Because no extractions or volume measurements are necessary, this technique is useful for field work. Its application to other species of animals, however, is questionable since it has been tested only on human subjects.

For purposes of variable dosing, 20 adult Wistar rats were divided into three groups. Group I consisted of eight females given an initial, one-time dose of 50 mg/kg lead acetate. Group II was comprised of eight females given a daily dose of 10 mg/kg lead acetate. Group III was made up of four males given an initial dose of 50 mg/kg lead acetate, followed by daily doses of 10 mg/kg. The lead acetate was prepared from a reagent grade chemical and nonsterile, deionized water at a concentration of 3 mg/ml. Doses were administered intraperitoneally with a 26-gauge needle.

Before dosing a background sample was obtained from each animal. The animals were maintained on tap water and rat chow (ad libitum). Each rat inhabited a single cage in a constant temperature room with a 12-hour light-dark cycle.

Blood samples were collected weekly by cardiac puncture. For this procedure the rats were anesthetized with 50 mg/kg sodium pentobarbital. Because of the lengthy recovery period from this drug, a barbituate, Mikedimide®, was administered after the sample had been obtained. Blood was collected in heparinized 1-cubic-centimeter (cc) tuberculin syringes and analyzed on the same day. Heparinized capillary tubes were filled from the syringes for hematocrit determinations. The Hematofluorometer was calibrated according to hematocrit results before sample analysis. The analytical technique was that prescribed by the manufacturer's manual.

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Avian Monitoring

The experimental design relating to feeding and dosing of lead in avian species has been reported by Edens et al. (1976). The study conducted at North Carolina State University, Raleigh, North Carolina, utilized Japanese quail. The quail received a nutritionally complete quail starter-grower diet to which lead in the form of lead acetate was added in levels of 0.1, 10, or 100 ppm. The amount of lead added was corrected for acetate moeity. Quail chicks consumed the experimental diet ad libitum from the time they were hatched.

When the quail were 6 weeks old, all experimental diets were changed to consist of a quail layer ration with added lead. The quail were sacrificed at 6 and 12 weeks of age and femurs were collected from both males and females. Eggs were collected throughout the study and subsequently shipped to the Environmental Monitoring Systems Laboratory. Preparation of the egg and femur samples for analysis consisted of weighing the sample and using a wet ashing procedure as described in our study of rats. Stable lead was determined by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

RODENTS

Excretion and Tissue Distribution in Rats Following a Single Oral Administration of Lead-210

The average daily excretion percentages are shown in Figures 1 and 2. The peak excretion value occurred at the first collection 24 hours following administration. The mean percent of administered dose recovered in the urine after three days was 0.160 with a standard deviation of ±0.088, and after 12 days it was 0.269 ± 0.168. The percentage of the dose recovered in the feces was 72.1 ± 13.0 and 89.0 ± 4.91 at 3 and 12 days respectively. Twelve days following oral administration, an average of $8.19 \times 10^{-3} \pm 4.62 \times 10^{-3}$ percent of the dose was found in the liver, whereas bone contained $4.33 \times 10^{-2} \pm 2.22 \times 10^{-2}$ percent of the ^{210}Pb.

Excretion and Tissue Distribution of Lead-210 Following Oral and Intravenous Administration to Ligated and Nonligated Rats

As shown in Table 3, 3 days following a single oral administration of ^{210}Pb as the nitrate, to rats having intact bile ducts, about 0.1 percent of the lead had been excreted in the urine, while about 95 percent was excreted in feces. The amount of the dose recovered in the tissues was about 5 percent. After a similar dose to rats with ligated bile ducts, about 0.1 percent was excreted in the urine and about 93 percent in the feces. The tissues maintained about 7 percent of the dose. The difference between the percentages found in the tissue indicates that about 2 percent of the dose was retained in the bile of the ligated rats.

Following intravenous administration of ^{210}Pb as the nitrate, rats with ligated bile ducts excreted about 4 percent in the urine, while those with intact bile ducts excreted about 6 percent. Only 7 percent of the dose was excreted via the feces of ligated rats as compared to 24 percent by intact rats. The amount of the dose recovered in the tissues was also different, with 71 percent recovered in rats with intact bile ducts versus 88 percent in rats with ligated bile ducts. If the differences in these figures are caused by lead in the bile, then as much as 20 percent of the ^{210}Pb dose would have been accumulated in the bile during the 3 days following intravenous administration; however, only 2 percent of the oral dose (or about 28 percent of the lead absorbed) was accumulated in bile during the same time period.

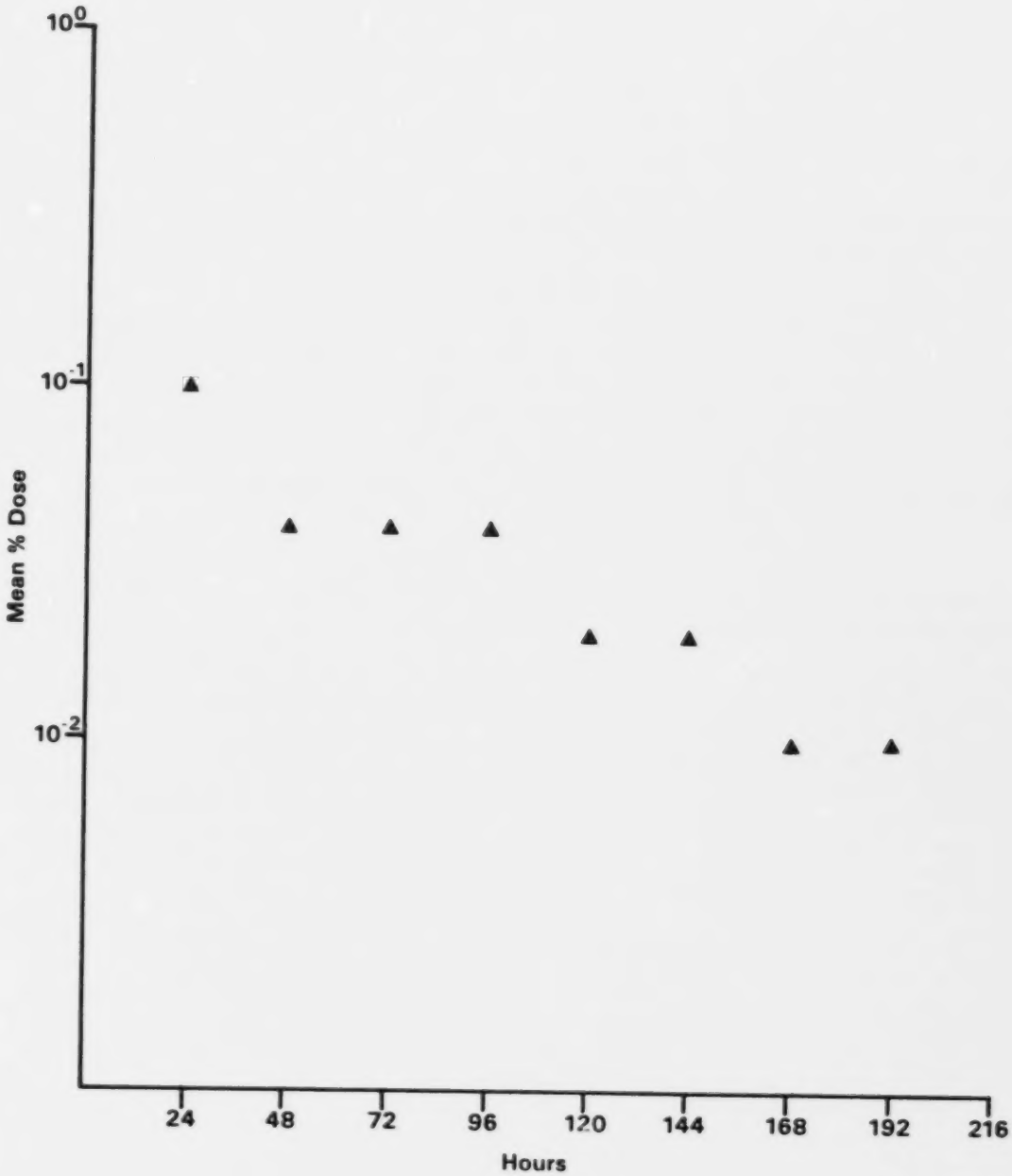

Figure 1. Mean percent of dose excreted daily in urine of rats following a single oral administration of lead-210.

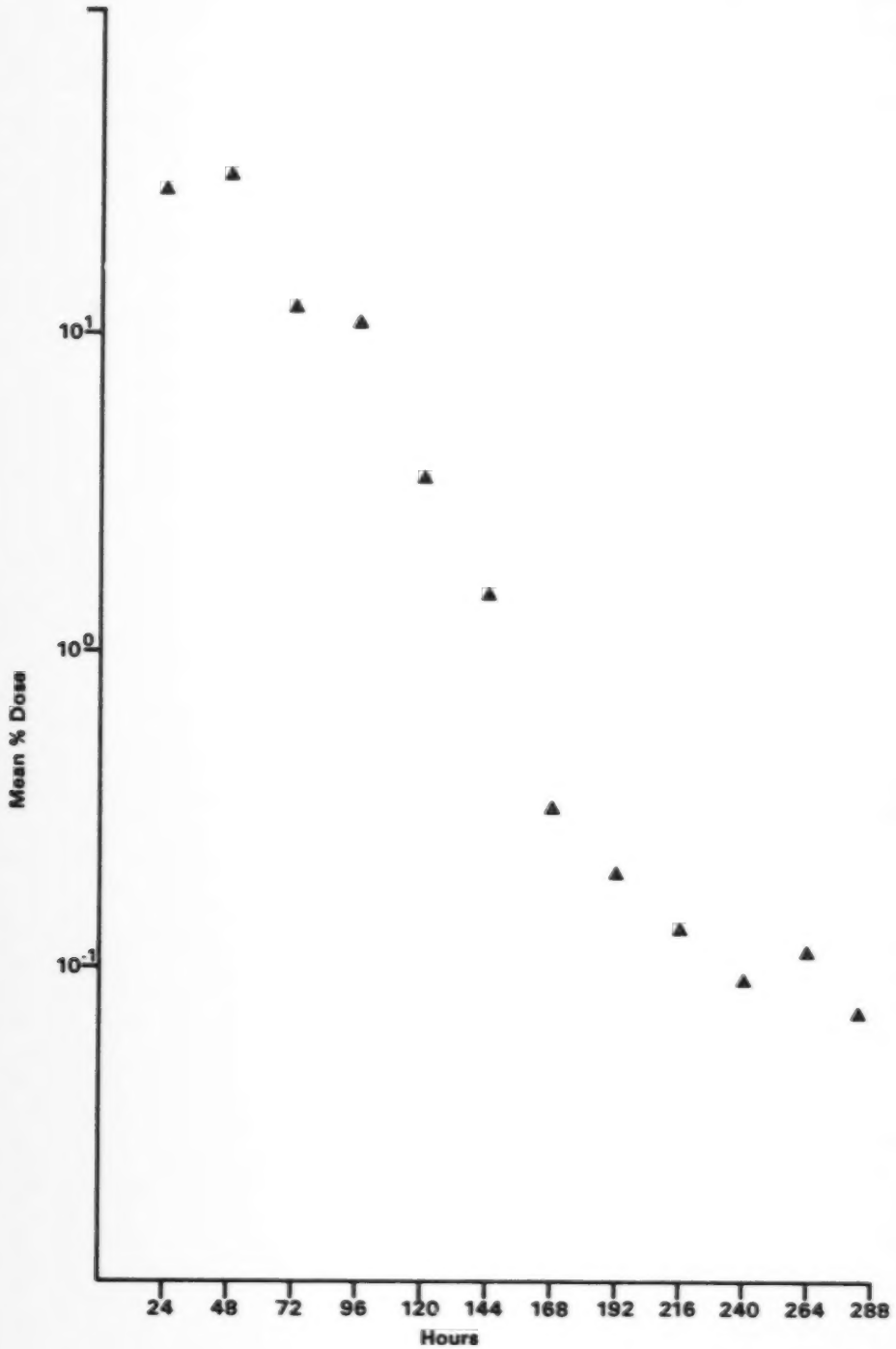

Figure 2. Mean percent of dose excreted daily in feces of rats following a single oral administration of lead-210.

TABLE 3. MEAN PERCENT OF ADMINISTERED DOSE RECOVERED FROM RATS

Measurement	Oral Administration				I.V. Administration	
	Non-Ligated	Non-Ligated	Non-Ligated	Ligated	Non-Ligated	Ligated
Number of rats	12	12	6	6	6	6
Days after administration	12	3	3	3	3	3
Age in days	100	100	51	51	52	52
Urine:						
Mean	0.269	0.160	0.098	0.102	5.86	4.09
Std. deviation	0.168	0.088	0.060	0.635	6.48	3.23
Std. error	0.053	0.028	0.024	0.251	2.65	1.32
Feces:						
Mean	89.0	72.1	95.1	92.8	24.1	6.75
Std. deviation	4.91	13.0	3.00	2.77	9.50	7.35
Std. error	1.55	4.12	1.22	1.13	3.88	3.00
Tissue:						
Mean	--	--	4.79	7.14	70.6	88.2
Std. devation	--	--	2.97	2.77	13.0	9.14
Std. error	--	--	1.21	1.13	5.31	3.73

Cikrt (1972) found that 6.7 percent of intravenously administered ^{210}Pb was excreted into bile during the first 24 hours following administration, as measured by cannulation of the bile duct of adult, 200-g rats, and collection of the excreted bile. Klaassen (1973) measured 1.0 µg/min/kg of lead in bile, 2 hours after intravenous administration to rats. A five times greater affinity of lead for liver than for plasma was found. Biliary excretion is temperature dependent, rising as the ambient temperature rises. It was also found by Klaassen that there were definite species differences in lead excretion in bile. Rabbits excreted less than one-half of the lead that was measured in the bile of rats, where dogs excreted less than one-fiftieth that of rats.

Figures 3 and 4 show the mean excretion values for ligated and intact rats following a single acute dose of lead nitrate. In Figure 3 the excretion values shown for urine after oral dosing were similar and therefore they were averaged for graphic representation. The percentage of lead excreted in the urine appeared to be very nearly a constant value over the period of the study.

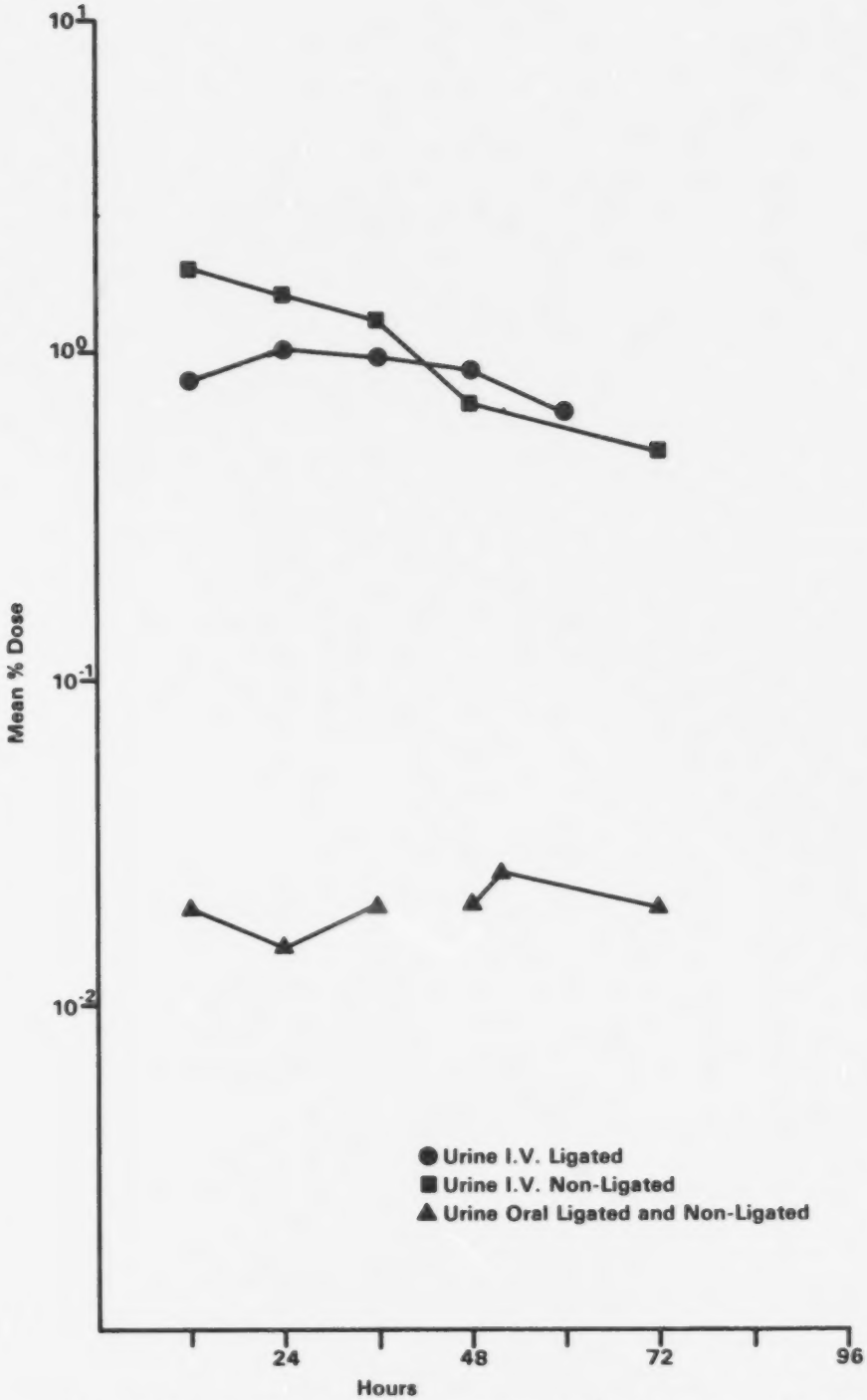

Figure 3. Mean percent of dose excreted daily in urine of rats with ligated or intact bile ducts following either oral or intravenous administration of lead-210.

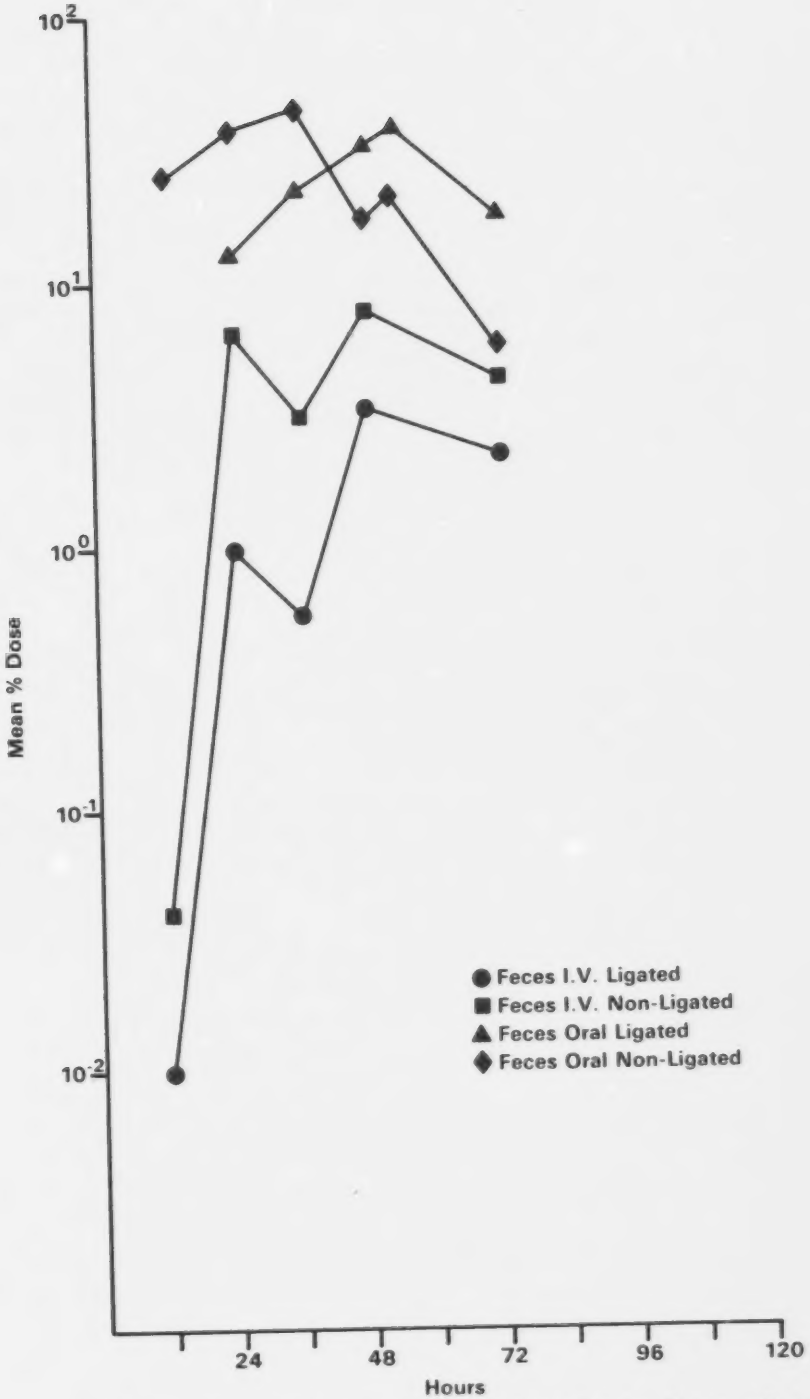

Figure 4. Mean percent of dose excreted daily in feces of rats with ligated or intact bile ducts following either oral or intravenous administration of lead-210.

Excretion of lead via the feces, exhibited unique curves dependent on the secretion of bile into the intestine. The percentage of ingested lead excreted in feces from rats having intact bile ducts peaked at 36 hours, then decreased rapidly. Lead in feces from rats with ligated bile ducts did not peak until 50 hours after dosing. These values seem to indicate that not only did the ligation of the bile duct decrease the overall percentage of lead excreted, but it also appeared to increase the time that the ingested lead remained in the gastrointestinal tract. The urine from ligated rats contained a higher percentage of lead at 50 hours after dosing than did the intact rats.

Figure 4 illustrates the excretion curve for the intravenously dosed rats. The primary difference between the curves for the rats having either ligated or intact bile ducts was the increased percentages of lead excreted in the feces of the intact rats, although the differences in the amount of lead excreted became less pronounced in samples obtained more than 48 hours following dosing. The percentage of dose excreted by the intact rats approached a peak 24 hours following dosing then dropped 12 hours later; this drop also occurred in the ligated rats. The peak value for the ligated rats occurred 48 hours after dosing. The urine curve for the intact rats appears to be a typical excretion curve following intravenous dosing. The urine curve for the ligated rats appears similar to that of an orally dosed animal. This may be due to the constant reabsorption of the bile by the ligated animals and slow excretion in the urine.

As shown in Table 4, the average percentages of the dose recovered in the various organs indicate that the expected increase in liver concentration of ligated rats occurred. The amount of lead concentrated in the kidney of ligated rats was higher than that of intact rats, following intravenous administration. The spleens of ligated rats contained a greater amount of lead than did the spleens of intact rats following oral administration.

Table 5 lists the percentages of lead recovered per gram of rat tissue or compartment. Following intravenous administration, the kidneys of ligated rats still contain more lead per gram than those of intact rats. This may indicate reabsorption of the ligated bile and excretion via the kidneys so the amount of lead accumulated in the bile may be higher than is indicated from a simple subtraction of tissue content.

The action of bile in the absorption and excretion of heavy metals appears to be very complex. Durbin (1972) reported that excretion of plutonium by humans was by way of the bile and digestive juices, and that fecal excretion of plutonium was decreased by one-half or more in persons whose gastrointestinal tracts were judged to be not normally stimulated. Ballou and Hess (1972) found that 50 percent of the plutonium excreted into the perfused intestine of the rat arrived by way of the bile during the first hour after plutonium injection. After treatment with DTPA the proportion excreted in bile was increased to 75 percent and the bile plutonium concentration increased 15 to 20 fold. Barth and Mullen (1974) using an artificial cow rumen have found that the heavy metal transuranics are dissolved by bile in the duodenum. Whether the heavy metals became more soluble due to complexing and whether the complex metal ions are more or less available for reabsorption was not definitely established.

TABLE 4. MEAN PERCENT OF DOSE TO RATS RECOVERED PER ORGAN

Measurement	Oral Administration		I.V. Administration	
	Ligated	Non-ligated	Ligated	Non-ligated
Liver:				
Mean	6.18×10^{-2}	3.05×10^{-2}	5.61	1.97
Std. deviation	1.94×10^{-2}	3.47×10^{-2}	7.92	2.13
Std. error	7.93×10^{-3}	1.42×10^{-2}	3.23	8.70×10^{-1}
Spleen:				
Mean	6.41×10^{-3}	6.62×10^{-4}	3.71×10^{-1}	5.46×10^{-1}
Std. deviation	5.06×10^{-3}	3.62×10^{-4}	5.01×10^{-1}	1.16
Std. error	2.26×10^{-3}	2.09×10^{-4}	2.04×10^{-1}	4.74×10^{-1}
Kidney:				
Mean	3.70×10^{-2}	5.85×10^{-2}	3.11	1.13
Std. deviation	1.43×10^{-2}	5.02×10^{-2}	2.29	1.28
Std. error	5.82×10^{-2}	2.05×10^{-2}	9.34×10^{-1}	5.23×10^{-1}
Heart:				
Mean	8.97×10^{-4}	7.72×10^{-4}	9.78×10^{-3}	5.71×10^{-3}
Std. deviation	5.02×10^{-4}	5.53×10^{-4}	8.44×10^{-3}	4.22×10^{-3}
Std. error	2.90×10^{-4}	3.19×10^{-4}	3.45×10^{-3}	1.89×10^{-3}
Brain:				
Mean	1.40×10^{-3}	5.06×10^{-4}	2.20×10^{-2}	1.57×10^{-2}
Std. deviation	5.86×10^{-4}	7.52×10^{-4}	1.67×10^{-2}	1.16×10^{-2}
Std. error	4.14×10^{-4}	3.76×10^{-4}	6.83×10^{-3}	4.74×10^{-3}
Skull:				
Mean	1.58×10^{-1}	9.16×10^{-1}	2.56	1.92
Std. deviation	3.50×10^{-1}	1.38	2.25	1.77
Std. error	1.56×10^{-1}	6.91×10^{-1}	9.19×10^{-1}	7.21×10^{-1}
Bone:				
Mean	9.70×10^{-1}	1.68×10^{-1}	1.36×10^{-1}	1.23×10^{-1}

The rapid excretion of lead with the bile during the first 72 hours after dosing may decrease as the lead is more tightly bound to the hepatocytes. Most lead carried in the blood is attached to the red blood cells (Goodman and Gilman 1970), therefore little is filtered when lower levels of lead are present. During exposure to high acute lead levels, a greater portion is filtered, so that biliary excretion and urinary excretion is at first rapid, then falls to a more steady state. Castellino and Aloj (1964) found that ^{210}Pb is rapidly distributed in the tissues of rats, the highest concentrations being in the kidneys, liver, and bones. Lead carried in the

TABLE 5. MEAN PERCENT OF DOSE TO RATS RECOVERED PER GRAM OF ORGAN OR TISSUE

Measurement	Oral Administration		I.V. Administration	
	Ligated	Non-ligated	Ligated	Non-ligated
Femur:				
Mean	4.67×10^{-2}	6.62×10^{-2}	1.12	1.10
Std. deviation	3.39×10^{-2}	3.33×10^{-2}	1.15	9.50×10^{-1}
Std. error	1.39×10^{-2}	1.36×10^{-2}	4.71×10^{-1}	3.88×10^{-1}
Skull:				
Mean	9.19×10^{-2}	1.91×10^{-1}	8.28×10^{-1}	5.48×10^{-1}
Std. deviation	7.23×10^{-2}	2.79×10^{-1}	6.64×10^{-1}	4.71×10^{-1}
Std. error	3.23×10^{-2}	1.25×10^{-1}	2.71×10^{-1}	1.92×10^{-1}
Liver:				
Mean	5.79×10^{-3}	4.86×10^{-3}	5.29×10^{-1}	1.78×10^{-1}
Std. deviation	3.49×10^{-3}	7.72×10^{-3}	6.20×10^{-1}	1.84×10^{-1}
Std. error	1.43×10^{-3}	3.15×10^{-3}	2.53×10^{-1}	7.52×10^{-1}
Spleen:				
Mean	3.44×10^{-3}	1.35×10^{-3}	6.15×10^{-1}	5.69×10^{-1}
Std. deviation	2.09×10^{-3}	1.28×10^{-3}	8.69×10^{-1}	1.15
Std. error	1.21×10^{-3}	7.40×10^{-4}	3.55×10^{-1}	4.70×10^{-1}
Kidney:				
Mean	2.85×10^{-2}	3.61×10^{-2}	1.93	8.58×10^{-1}
Std. deviation	2.01×10^{-2}	3.23×10^{-2}	1.48	7.37×10^{-1}
Std. error	8.21×10^{-3}	1.32×10^{-2}	6.06×10^{-1}	3.01×10^{-1}
G.I. Track:				
Mean	2.66×10^{-1}	1.55×10^{-1}	1.92×10^{-1}	2.80×10^{-1}
Std. deviation	8.71×10^{-2}	1.72×10^{-1}	2.18×10^{-1}	3.09×10^{-1}
Std. error	3.56×10^{-2}	7.03×10^{-2}	8.90×10^{-2}	1.26×10^{-1}
Muscle:				
Mean	4.39×10^{-4}	6.39×10^{-4}	2.90×10^{-2}	1.63×10^{-2}
Std. deviation	4.58×10^{-4}	1.14×10^{-3}	2.41×10^{-2}	1.39×10^{-2}
Std. error	2.05×10^{-4}	5.68×10^{-4}	9.82×10^{-3}	5.69×10^{-3}

blood is deposited in bone as relatively insoluble tertiary lead phosphates which are removed from bone tissue at a rather slow rate. Neathery and Miller (1972) found that most lead entering the systemic circulation by injection in rats invades the reticuloendothelial system represented by bone marrow, spleen, and liver. In contrast, the lead entering the gut wall goes to the bone and kidney of rabbits. Blaxter (1950) found that in other species most orally ingested lead is deposited in the skeleton. Thus, lead may initially be concentrated in bone until a possible threshold is reached and then deposition is mainly in the kidney, where turnover rate is slow.

As shown in Table 4, bone was the primary deposition site for lead in rats. The skull and femurs were selected as representative of the skeleton of the rat. Data for total bone were calculated assuming 10 percent of the body weight of the adult rat is represented by the skeleton (Sikov and Mahlum 1972).

Holtzmann (1963) measured radium D (^{210}Pb) concentration in bone and found that it is higher in trabecular than cortical bone, although the skull appeared to be the bone most representative of total skeleton. Based on five human subjects, the mean deviated by 16 percent. Tibia, mandible, and joint bone were lower than average and rib was higher (38 ± 34 percent above average). This agreed well with Kehoe (1961), who found that the concentration of lead, after recent exposure, was often higher in the flat bones than in the long bones.

Holtzmann (1963) concluded that within a factor of 2, radium D is uniformly distributed in human skeletal mineral, and the total skeletal content of radium D may be estimated with some assurance from measurements on a single bone.

It is interesting to note, as shown in Table 5, that the percentages of dose recovered in the skull versus the femur were higher in orally dosed rats. In intravenously dosed animals the chemical form may be different from that of lead absorbed through the gut and a higher percentage could be deposited in bone marrow.

The total percent of ^{210}Pb retained in the tissue was found to be about 5 percent in 51-day-old rats, 3 days after an acute oral ingestion. This agrees with values found by other authors as noted by Karhausen (1973). During the same time period, 70 percent of an intravenously administered ^{210}Pb dose was retained.

The standard error was found to be relatively high in all measurements of lead metabolism. This has also been found to be true by other authors, as noted by Di Ferranti and Bourdeau (1973) and seems to indicate that storage of lead in different compartments is far from being consistent. Absorption of lead appears to be affected by dietary calcium, chemical form, Vitamin D intake, diet composition, load of lead and gastric acidity (Karhausen 1973).

Placental and Milk Transfer of Stable Lead

The pregnant rat females receiving water containing 50 ppm of lead ingested an average of about 30 ± 10 ml per day, approximately 1.5 mg of lead per day, or a total of about 190 mg of lead prior to parturition.

At parturition, 12 days following initial dosing, a minimum of two neonates per group, or enough to decrease the rats litter size to six, were sacrificed and the lead content of the whole body was determined. The average amount of lead recovered from the whole bodies of 19 neonates was 6 µg of lead per neonate (see Table 6). This is about 3×10^{-5} percent of the total lead ingested by the dams or about 4×10^{-2} percent of the lead absorbed. An average of one mg of lead per offspring or 6×10^{-3} percent of the dose, was present in neonates from mothers who received the same single dose (50 mg/kg) of lead but did not ingest a daily lead supplement to their drinking water. The mean weight of neonates from rat dams ingesting lead daily was no different from those of neonates born from dams ingesting a single dose of lead and fell within the mean weights, 5 to 6 g, for neonates of this breed (Ralston Purina Company 1961).

The lead content of the neonates may be different than it would have been had the neonates been sacrificed immediately after birth. In most cases the dams gave birth during the night and the young were not removed until the following morning. This introduced at least two possible errors. First, the young were suckling during this time, a maximum of 16 hours and excretion of lead may have increased due to mobilization of lead in soft tissues shortly after birth. How much of this mobilized lead is excreted and how much stored in bone is not known. Singh et al. (1976) showed that the kidney, liver, heart, and brain of newborn rats contained very high amounts of lead when the young were sacrificed within half an hour after birth; a very significant reduction occurred after 1 day with further reduction after 7 days, however, blood levels remained almost identical to the levels obtained 1 day after birth. No bone samples were taken so it is not known if increased deposition occurred at this site or if the lead was secreted in the bile and excreted via the feces.

Other authors have found lead to be rapidly transported to the fetus. (Hubermont et al., 1976; Schaller et al., 1976; McClain and Becker 1975; Kostial and Momcilovic 1974; Green and Gruener 1974; and The Task Group on Metal Accumulation 1973).

Green and Gruener (1974) found that lead transport was so rapid that the fetus is in equilibrium with the mother rat 24 hours after injection, although other authors, Schaller et al. (1976) and McClain and Becker (1975), indicate that the placenta acts as a diffusion barrier and limits the passage of lead to the fetus since large maternal-fetal concentration gradients existed.

Results from the analysis of hair samples are listed in Table 7. Since rats are born without hair the first samples were obtained at weaning, about 21 days after birth. The transfer of lead from dam to young during lactation has been previously established. Green and Gruener (1974) found that significant amounts of lead were transferred to the nursing rats even a week after a

TABLE 6. PLACENTAL TRANSFER OF LEAD IN RATS

Number of Neonates Sacrificed	Mean Weight* per Neonate (g) Mean S.D.	µg of Lead* per Gram Whole Body	µg of Lead* per Neonate
Experimental:**			
6	5.70 ± 0.46	1.76 ± 0.60	6.75 ± 4.80
5	5.93 ± 1.94	0.88 ± 0.53	5.20 ± 3.09
2	5.79 ± 0.65	1.07 ± 0.18	6.25 ± 1.80
2	5.93 ± 0.26	0.84 ± 0.04	5.00 ± 0.21
2	5.78 ± 0.87	0.88 ± 0.13	5.00 ± 0.45
2	6.00 ± 1.21	0.85 ± 0.16	5.00 ± 0.49
Mean	5.83 ± 0.49	1.19 ± 0.59	6.74 ± 3.16
Controls:			
8	5.99 ± 0.45	Background	Background
2	5.65 ± 0.50	0.22 ± 0.28	1.30 ± 1.70
2	5.68 ± 0.62	0.20 ± 0.21	1.14 ± 1.31
2	5.60 ± 0.59	0.45 ± 0.18	2.50 ± 0.71
Mean	5.69 ± 0.37	0.22 ± 0.21	1.24 ± 1.02

* Standard deviations are shown for each mean value.

** Dams exposed to lead in drinking water mean ± one S.D.

single administration. Kostial and Momcilovic in 1974 discovered that during pregnancy and lactation both lead-203 and calcium-47 were transferred in substantial amounts from mother to fetuses and offspring. The amount transferred was related to the physiological state, as that amount was higher in late lactation.

The results shown in Table 7 indicate that little lead was incorporated into the hair of young rats prior to weaning, and with the relatively low lead doses received by the animals in this study, the amount transferred to milk would be small. Stanley et al. (1971) found that less than 0.02 percent of a single oral dose of lead-203 was secreted in bovine milk over a 5-day period. Neathery and Miller (1972) compiled the results of a number of investigations into the secretion of lead into milk, all of which indicate that under most conditions, relatively little lead is secreted into milk.

However, lead absorbtion is much higher in newborn rats. Momcilovic and Kostial (1974) state that whole body retention of lead was higher in sucklings compared to adults. After an 8-day period there was still 85 percent of an injected dose remaining in the sucklings compared to 34 percent in the adult

TABLE 7. MEAN* LEAD CONTENT OF HAIR AND FEMUR FROM FEMALE AND YOUNG RATS AT VARIOUS TIMES (μg/g)

Animals	Treatment	Hair				Femur
		Parturition	Weaning	Weaning + 1 mo	Weaning + 2 mo	Weaning + 2 mo
3 dams (a, b, c)	Lead in drinking water	20.4 ± 2.30	0.26 ± 0.02	15.2 ± 7.62	19.8 ± 4.30	21.3 ± 5.92
3 litters (a, b, c)	Lead in milk and drinking water		0.36 ± 0.15	10.6 ± 3.0	11.7 ± 2.09	13.1 ± 2.36
2 dams (d, e)	Lead in drinking water	9.72 ± 4.91	0.62 ± 0.26	12.8 ± 2.26	10.6 ± 3.70	18.7 ± 6.79
2 litters (d, e)	Lead in milk only		0.24 ± 0.12	17.2 ± 1.89	10.6 ± 4.41	13.4 ± 1.94
2 dams (f, g)	No lead exposure	1.12 ± 0.22	0.23 ± 0.09	0.20 ± 0.05	0.28 ± 0.02	4.88 ± 1.14
2 litters (f, g, nursed by dams h, i)	Lead in milk and drinking water		0.52 ± 0.25	11.2 ± 5.02	17.6 ± 6.31	20.0 ± 4.38
2 dams (h, i)	Lead in drinking water	9.80 ± 3.68	0.27 ± 0.21	19.6 ± 2.19	16.6 ± 1.98	20.5 ± 1.70
2 litters (h, i, nursed by dams f, g)	No lead exposure from milk or water		0.60 ± 0.26	0.26 ± 0.13	0.32 ± 0.16	4.96 ± 2.03
Control dam	No lead exposure	2.14	0.63	0.49	0.55	7.41
Control litter	No lead exposure		0.34 ± 0.11	0.27 ± 0.10	0.26 ± 0.10	3.35 ± 1.20

* Standard deviations are shown for each mean value.

rats. Forbes and Reina (1972) investigating the effect of age on gastrointestinal absorption of lead found that peak lead absorption, 89.7 percent, occurred at 20 days of age, decreasing to 16 percent at 89 days. But of the amount of lead absorbed, preferential deposition would probably take place in the bone of the rapidly growing young with little being available for incorporation into hair.

Kostial and Momcilovic (1974) also found that the transplacental transport of lead was eight times lower than that of calcium while the transmammary transport of lead was only four times lower than calcium. During lactation, the retention of calcium-47 in femurs and teeth of the dams was 71 percent and 50 percent lower than control females, and lead retention during the same time was 33 percent and 24 percent lower. Therefore, during the stress of lactation, lead was not as available for incorporation into the hair of the dams as it would be during other physiological states.

This is confirmed in our study which shows the lowest amounts of lead in hair during lactation. One month after weaning, the lead content of hair from both dams and young receiving lead had increased significantly. The hair of offspring receiving milk containing lead but receiving no supplementary lead following weaning contained elevated levels 1 month after weaning, but the levels had started to drop after an additional month indicating that there was a delay between increased ingestion of lead at the latter stages of lactation and incorporation into hair. Litters from control dams receiving milk from lead-dosed foster dams gradually increased the amount incorporated in hair.

Litters receiving lead only during fetal development did not show significantly greater amounts of lead in their hair as compared to the hair of control litters.

Femurs collected 2 months after weaning showed the expected lead levels dependent on the exposure to lead. Since all dams received some lead (the control dams inadvertently received the initial oral dose of lead), the concentration of lead at levels exceeding background in their femurs and in those of their offspring was unexpected. The higher correlation of the amount of lead in the femurs of the young in the cross-fostered groups to that of their foster mothers rather than their natural mothers was unexpected. The correlation of lead in femurs of young remaining with their mothers was not as close, even in the control groups.

The results of the study indicate that analysis of hair for lead is a valid indication of past exposure but care would have to be taken as the uptake is affected by the physiological state of the animal.

The lead content of blood samples from dams and offspring exhibited such wide variability that treatment-related differences were absent. Blood samples were analyzed for zinc protoporphyrin content immediately after collection using the ZnP Hematofluorometer 4000. The results are shown in Table 8. If one assumes the ZnP results are valid for rats (see section on birds), it would appear that the equivalent protoporphyrin (see footnote to Table 8) values for the weanlings were higher than those for any time after weaning. As the results from the one control litter follows this same trend,

TABLE 8. ZINC PROTOPORPHYRIN LEVELS IN THE BLOOD OF RATS AND OFFSPRING

Animals	Treatment	µg EPP* per ml Blood			
		Dams 6 d Pregnant (background)	Weaning	Weaning + 1 mo	Weaning + 2½ mo (sacrificed)
3 dams (a, b, c)	Lead in drinking water	22 ± 6	20 ± 20	23 ± 7	21 ± 8
3 litters (a, b, c)	Lead in milk and drinking water		33 ± 13	10 ± 6	10 ± 4
2 dams (d, e)	Lead in drinking water	23 ± 3	12 ± 4	16 ± 1	8 ± 0
2 litters (d, e)	Lead in milk only		26 ± 6	13 ± 5	8 ± 2
2 dams (f, g)	No lead exposure	33 ± 18	30 ± 9	10 ± 5	12 ± 2
2 litters (f, g nursed by dams h, i)	Lead in milk and drinking water		35 ± 22	11 ± 6	10 ± 5
2 dams (h, i)	Lead in drinking water	18 ± 6	25 ± 16	16 ± 1	10 ± 4
2 litters (h, i nursed by dams f, g)	No lead exposure from milk or water		34 ± 9	15 ± 6	10 ± 1
Control dam	No lead exposure	47	25	17	33
Control litter	No lead exposure		29 ± 22	12 ± 5	14 ± 12

* EPP = equivalent protoporphyrin. The instrument measures the ratio between zinc protoporphyrin and hemoglobin in blood. Consequently, the raw data from the instrument is presented in ZnP per volume of red blood cells. The instrument is calibrated to convert the raw reading electronically to units of equivalent µg per 100 milliliters of whole blood at a fixed hematocrit to conform to the CDC definition of the Modified Piomelli Technique.

it is questionable if this was a result of the lead ingestion. On the other hand, if the EPP value for the control dam is correct then this animal was either exhibiting clinical lead poisoning as interpreted for humans (>40 µg/100 ml blood, U.S. Public Health Service 1971) or was anemic. Unfortunately the hematocrit for this blood sample was not obtained. However, the subsequent collection indicated a low hematocrit reading, which would indicate anemia during pregnancy and lactation. The blood collected from this animal after the young were weaned and one month after weaning showed that the hematocrit was normal based on the mean normal packed cell volumes listed by Schalm in 1965.

The EPP values of offspring receiving lead from either placental transfer, milk or water ingestion did not indicate a significant difference from the control offspring. The amount of lead actually available to the offspring was evidently too small to affect the ZnP values as determined by the ZnP Hematofluorometer 4000. (See also following section.)

It was hoped that the placental transfer study could be repeated using lead-poisoned dams and bucks from our own breeding colony but lack of personnel precluded repeating the study.

Analysis of Lead in Blood

Figure 5 shows the fluctuation in zinc protoporphyrin levels over the experimental period of 50 days. All data points reflect mean values for the animals sampled. Mean background levels for each group were all equal to or less than 10 µg EPP/100 ml blood. No appreciable increases in ZnP levels were noted for 12 days.

Within 20 days Group I showed an increase of 15 µg EPP/100 ml of blood. The zinc protoporphyrins then maintained a plateau for 18 days, followed by a rise to 20 µg EPP/100 ml of blood above background levels. At the conclusion of the study ZnP levels were still elevated 15 µg EPP/100 ml of blood over baseline.

For sampling purposes, Group II was divided into two subgroups of four, which were sampled on separate days. This procedure was also used on Group I and the data reflect a homogeneous group. The data for Group II, however, reveals separate levels of ZnP for each subgroup. While each subgroup reached an initial increase of approximately 20 µg EPP/100 ml of blood above background within 14 days, their subsequent courses diverged. One subgroup continued to rise to 50 µg EPP/100 ml of blood above background, at which point the animals died. The second subgroup showed a slight rise (5 µg EPP/100 ml of blood) and, after day 32 had decreasing levels of ZnP. Nevertheless, at the experiment's conclusion, subgroup II showed ZnP levels that were 20 µg EPP/100 ml of blood above background.

Group III showed a 20 µg EPP/100 ml of blood rise in 18 days. A plateau followed for 15 days, at which time there was another rise to 28 µg EPP/100 ml of blood above background.

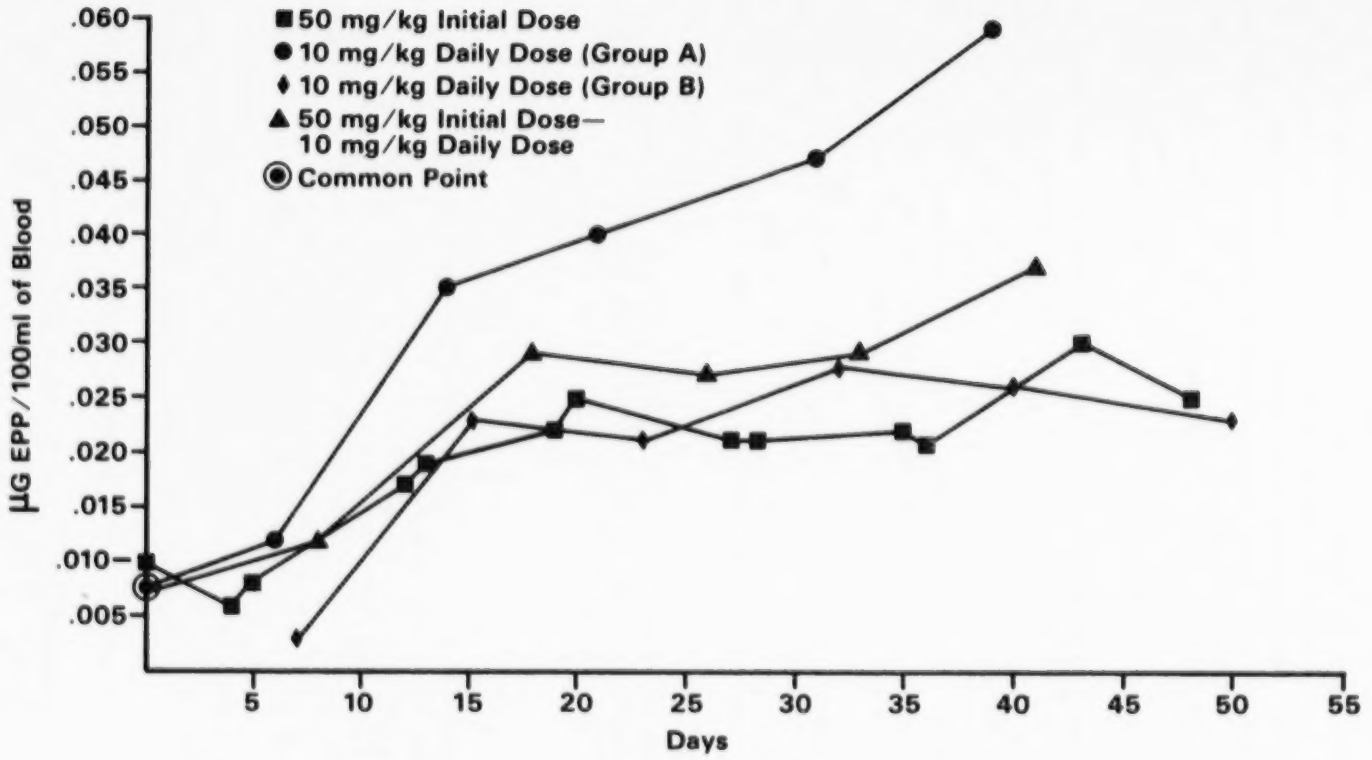

Figure 5. Micrograms of equivalent protoporphyrin per 100 ml of blood from rats receiving either acute or chronic doses of stable lead as the acetate.

From the data presented here it appears that the Hematofluorometer is able to detect lead in the rat, delivered in toxic doses, in a period of 12 to 18 days. The rise in zinc protoporphyrins of approximately 20 µg EPP/100 ml of whole blood is not so dramatic that it would be of significance without background levels with which to compare it. Of value is the fact that the measured effect has considerable longevity, although not as long as in humans. Zinc protoporphyrins, once formed, bind to the cellular membranes of red blood cells. Thus, the effect may be measured for the life of the red blood cell (60 to 70 days, Schalm 1965). The 2-week lag time between dosing and effect is presumably due to the time required for heme synthase enzyme inhibition to be translated into detectable levels of zinc protoporphyrins.

Large hematocrit fluctuations were observed in the rats. Lead is a known etiologic agent of anemia, but the possibility exists that frequent blood collection also contributed to this phenomenon. It is important to note that the Hematofluorometer yields a value based on the ratio of zinc protoporphyrins to hemoglobin within calibration standards established for humans. Thus, anemia should not affect the validity of these results in humans but could do so if the hematocrit values for the animal in question fell below standards supplied by the manufacturer.

Erthropoietic porphyria produces a porphyrin with different absorption and fluorescence maxima than zinc protoporphyrins and thus the Hematofluorometer will not confuse the two. It must be kept in mind, however, that the porphyrin produced by iron deficiency is identical to that produced by lead toxicity.

From the small amount of data gathered here it would appear that there are no appreciable differences between male and female rats with respect to zinc protoporphyrin detection of lead toxicity.

BIRDS

The mean lead content in six eggs from each dose group is shown in Table 9. The lead was concentrated mainly in the shell, but also occurred in the white and yolk portions of the quail eggs. The concentration of lead in all portions of the egg was dependent on the amount of lead in the diet of the laying hens. As it was not known precisely how much lead the hens ingested, a percentage of dose value could not be calculated, so it is not known if there is a maximum amount of lead deposited in eggs regardless of the availability.

Femurs from five quail in each dose group were averaged to obtain the mean values listed in Table 10. The femurs show definite correlation with the amount of lead consumed by the quail. These results agree with those obtained by Ohi et al. (1974), Tansy and Roth (1970), and Getz et al. (1977), who found a correlation between lead in femurs of birds and their location of residence.

It would appear that resident avian populations may provide a reliable biological indicator of an increase of lead in the environment of a specific area. Further work must be done to determine the minimal time between exposure to lead and detection of increased content in eggs or femurs.

TABLE 9. LEAD IN FEMURS FROM JAPANESE QUAIL CONSUMING LEAD ACETATE, FROM HATCHING TO 12 WEEKS OF AGE

Lead in Feed Consumed	µg Pb per g shell			µg Pb per shell		
	Mean	S.D.	S.E.	Mean	S.D.	S.E.
1 ppm	2.95	0.836	0.374	1.03	0.283	0.127
10 ppm	78.8	37.6	15.4	30.3	17.4	7.11
100 ppm	1,930	1,050	471	741	625	297
Control	2.17	1.74	0.780	1.16	0.660	0.295

S.D. = standard deviation
S.E. = standard error

TABLE 10. LEAD IN EGGS FROM JAPANESE QUAIL CONSUMING LEAD ACETATE, FROM HATCHING TO 12 WEEKS OF AGE

Lead in Feed Consumed	µg Pb per g shell			µg Pb per shell			µg Pb per g Egg White	µg Pb per g Egg Yolk
	Mean	S.D.	S.E.	Mean	S.D.	S.E.		
1 ppm	0.688	0.249	0.102	0.591	0.189	0.770	0.051	0.162
10 ppm	1.26	0.676	0.302	0.794	0.375	0.168	0.083	0.274
100 ppm	2.87	1.00	0.408	2.07	0.581	0.237	0.767	0.873
Control	0.346	0.266	0.109	0.242	0.191	0.078	0.030	0.130

S.D. = standard deviation
S.E. = standard error

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